

# **SMALL PEPTIDES HAVING ANTI-ANGIOGENIC AND ENDOTHELIAL CELL INHIBITION ACTIVITY**

## **FIELD OF THE INVENTION**

The present invention involves small peptides that function as potent angiogenic inhibitors. In particular, the invention relates to small peptides that can be synthesized by enzymatic or chemical methods. In addition, the invention relates to use of the small peptides to inhibit angiogenesis, to treat angiogenesis-related diseases, and to inhibit endothelial cell proliferation and reduce tumor growth.

## **BACKGROUND OF THE INVENTION**

Angiogenesis is the process of new blood vessel formation from pre-existing vessels, involving endothelial cell proliferation, migration and assembly into tubule structures (31). It plays important roles in many normal physiological functions such as embryonic development and wound healing (1). In addition, inappropriate angiogenesis is also associated with various pathological conditions including solid tumor growth and metastasis, rheumatoid arthritis and psoriasis (2). Many molecules that inhibit tumor angiogenesis have been shown to inhibit tumor growth including antibodies against angiogenic factors, natural and synthetic compounds that inhibit angiogenesis, and the natural angiogenic inhibitors like the angiostatin and endostatin proteins produced by tumor cells (3-8). Anti-cancer therapy by inhibiting tumor angiogenesis is called anti-angiogenic therapy and has shown great potential as an effective new method for treating cancer, especially solid tumors (9).

Plasminogen is a plasma glycoprotein synthesized mainly in the liver. It is the precursor of the serum protease plasmin that plays important roles in the fibrinolytic system and clot dissolution (10). At the amino terminus, plasminogen contains five homologous repeats that form looped "kringle" structures held together by disulfide bonds. Plasminogen

binds to fibrin through lysine binding sites located on the five kringle domains (k1 through k5) (10). Each kringle domain is about 80 amino acids in length and different kringle domains are highly homologous to each other in amino acid sequences. A naturally occurring fragment of plasminogen containing the first four kringle domains (k1-k4) has been isolated from serum and urine of mice bearing a low metastatic Lewis lung carcinoma. This plasminogen fragment has been named angiostatin (equivalent to amino acids 98-440 of murine plasminogen) and is a potent angiogenesis inhibitor that can inhibit endothelial cell proliferation as well as tumor growth and metastasis in mice with no obvious toxicity (5,6,11). Furthermore, recombinant individual kringle domains or their combinations expressed in *E. coli* have been found to be able to inhibit endothelial cell proliferation to various degrees with k5 the most potent, followed by k1 and k3 (12,13). The k5 domain is not present in the naturally existing angiostatin protein but recombinant k5 also functions as an angiogenic inhibitor by inhibiting endothelial cell proliferation, migration as well as inducing cell cycle arrest and apoptosis (13,32,33). All these studies indicated that the integrity of the kringle structures and its proper folding are critical in maintaining the kringle domain's as well as angiostatin's functions as angiogenic inhibitors.

Endostatin is a protein first identified from a hemangioendothelioma cell line in 1997 (17). It is a 20kDa C-terminal fragment of collagen XVIII, a novel collagen that consists of a N-terminal region, a series of collagen-like domains with interruptions and a 35kDa C-terminal noncollagenous domain (18,19,20). Recombinant endostatin functions as a potent angiogenesis inhibitor in vitro as well as in vivo (17). Systemic administration of endostatin to tumor bearing mice regressed the primary tumor without inducing drug resistance (21). Recently, endostatin was found to be a zinc-binding protein and the zinc-binding is essential for its antiangiogenic activity (22,23). Human clinical trials of endostatin started in September, 1999.

Vascular Endothelial Growth Factor (VEGF) is a potent endothelial specific mitogen. VEGF functions through two high affinity tyrosine kinase receptors: FLT-1 or Vascular Endothelial Growth Factor Receptor Type 1 (FLT-1/VEGFR1) and FLK-1, also known as KDR or Vascular Endothelial Growth Factor Receptor Type 2 (FLK-1/VEGFR2) (26). Both receptors are specifically expressed in endothelial cells. FLT-1 and KDR/FLK-1 stimulate endothelial cell proliferation and migration by binding to these two tyrosine kinase receptors (24). VEGF is also known as vascular permeability factor due to its ability to induce vascular leakage (24). The ligand binding domains of the two receptors as well as the receptor binding sites of VEGF have been studied by site-directed mutagenesis and X-ray crystallography (25-28). VEGF binds its two receptors through different amino acid contacts (25). The first three immunoglobulin loops of the FLT-1 receptor seem to be the main area responsible for VEGF binding (29,30). The signal transduction triggered by VEGF through its receptors play critical roles in both physiological angiogenesis as well as pathological angiogenesis such as solid tumor growth and metastasis by stimulating embryonic angiogenesis and tumor angiogenesis. An anti-VEGF monoclonal antibody has been shown to inhibit tumor growth in mice by reducing the vessel density of the tumor (14). Likewise, trans-dominant mutants of both receptors have been shown to inhibit tumor growth in mice (15).

Protein-protein interactions are crucial to many physiological and pharmacological processes. They are specific and exhibit high affinity interactions due to molecular recognition sites found on the surface. It has also been observed that proline residues are sometimes found at the ends of the linear sequences that constitute the site of a protein-protein interaction. It has been shown that the probability of finding proline residues in the flanking segments of the protein-protein interaction sites is 2-3 times that of their random distribution. And the proline residues are not normally present within the interaction sites, but in the flanking

segments. They are not directly involved in the interaction between proteins (14,15).

#### SUMMARY OF THE INVENTION

5 The invention provides compositions comprising the peptides that are effective in inhibiting undesirable angiogenesis. The invention includes small peptides that have the ability to inhibit bovine aorta endothelial cell proliferation in the presence of basic Fibroblast Growth Factor (bFGF) in vitro. They can also inhibit angiogenesis in the chick  
10 chorioallantoic membrane (CAM) in vivo. The peptides of the invention are typically less than 20 amino acids in length. They preferably contain proline residues at each end or penultimate thereto. Peptides of the invention can be identified within the kringle domains of plasminogen or within the amino acid sequences of endostatin, VEGF, and VEGF  
15 receptors, especially FLT-1 and KDR/FLK-1.

Some preferred peptides of the invention are named Angio-1, Angio-2, Angio-3, Angio-4 and Angio-5 (SEQ. ID. NOS. 1-3, 11, and 12) according to the plasminogen kringle domains they are derived from. Other preferred peptides are shown in Table 1 (SEQ ID NOS. 29-50).

20 Small peptides from homologous regions of kringle 1 to kringle 5 have been designed. In accordance with this invention, such small peptides can function as angiogenic inhibitors in inhibiting chick CAM angiogenesis. Furthermore, one of the peptide Angio-3 derived from kringle 3, can inhibit BAE cell proliferation as well as tumor growth in  
25 nude mice. This finding is in direct contrast to the previous observation that the integrity of the kringle structures is required to maintain its anti-endothelial function.

Methods for preventing or treating undesirable angiogenesis, for example to prevent tumor metastasis or inhibit the growth of a primary  
30 tumor, by administration of compositions of the anti-angiogenic peptides, are also within the scope of the invention.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Inhibition of BAE cell proliferation by peptide Angio 3.

Figure 2. Inhibition of BAE cell proliferation by peptides Angio-2  
5 and Angio-4.

Figure 3. Inhibition of chick CAM angiogenesis on 4 day embryo by Angio-3 at 120 ug/cover slip. The arrow indicates the inhibition of the growth of new vessels at the site of peptide application.

Figure 4. Inhibition of chick CAM angiogenesis on 4 day embryo by  
10 Angio-4 at 120 ug/cover slip. Arrows indicate the inhibition of the growth of new vessels at the site of peptide application.

Figure 5. Inhibition of chick CAM angiogenesis on 10 day embryo by Angio-2 at 12 ug/cover slip.

Figure 6. Control cover slip containing PBS on 10 day old chick  
15 embryo CAM. The arrow indicates the position of the control cover slip on CAM.

Figure 7. Inhibition of BAE cell proliferation by peptides Endo-1, Endo-2, Endo-3 and Endo-4.

Figure 8. Inhibition of chick CAM angiogenesis in 10 day embryo  
20 by Endo-4 at 1.8 µg/cover slip.

Figures 9A-9B. Inhibition of chick CAM angiogenesis in 13 day embryo by mVEGF peptide at various doses as indicated. Arrows indicate the inhibition of growth of new vessels at the site of peptide application. Fig. 9A - 0 hrs incubation; Fig. 9B - 40 hours incubation.

Figure 10. Antiangiogenic effect of peptides in the chick CAM  
25 assay. The number of positive inhibitions over the number of CAMs examined for each group are indicated above each bar.

Figure 11. Antiangiogenic effect of peptides in the chick CAM  
30 assay. The number of positive inhibitions over the number of CAMs examined for each group are indicated above each bar.

Figure 12. Inhibition of tumor growth in mice by peptide angio-3.

## DETAILED DESCRIPTION OF THE INVENTION

Small peptides were designed according to the amino acid sequences of the kringle domains of human plasminogen or from the amino acid sequences of endostatin, VEGF, FLT-1 or KDR/FLK-1 using the "proline bracket model" described in references 14 and 15. The peptides were synthesized using a peptide synthesizer (Applied Biosystems, Inc., model 473A). The peptides were then purified by typical HPLC methods using a C18 reverse-phase column and eluting with an acetonitrile gradient.

Angiostatin-derived peptides of the present invention represent a portion of a kringle domain of plasminogen. The effective portion of a kringle domain is generally residues 30 to 38 or 39 of a kringle domain, numbered as in reference 13. However, it is acceptable to add additional amino acids to this generally effective core peptide. Thus, peptides representing residues 27 to 40 or 41 of a kringle domain would be expected to be effective. Preferred peptides of the invention are derived from human or mouse plasminogen. The exemplified peptides of SEQ. ID. NOS.: 1-3, 11 and 12 represent residues 29-38 or 29-39 of human plasminogen kringle domains (13).

The peptides of the invention preferably range from 8 to 20 amino acids long. Peptides of the invention typically range from 7-20, more typically from 10-15 amino acids in length. The peptides are often about 10 amino acids in length. The peptides are more preferably 8, 9 or 10 residues long, most preferably 10 residues long.

The peptides of the invention may contain a single cysteine residue. In such a case, it is desirable that the cysteine residue be derivatized to block disulfide bond formation. Most preferably, peptides of the invention lack cysteine residues altogether.

The peptides also preferably contain a pair of proline residues. Each proline residue of the pair is preferably the residue penultimate to a terminus of a peptide, but either or both of the proline residues can be a terminal residue. Imino acids similar to proline, in that their side chains

form a ring containing the peptide bonding nitrogen and  $\alpha$ -carbon, and that will "break" an  $\alpha$ -helix or  $\beta$ -sheet secondary structure, can be substituted for one or more of the proline residues. Exemplary peptides are shown as SEQ. ID. Nos. 1-50. The more preferred peptides are those of SEQ. ID.

5 NOs. 1-3, 11, 12, 29-33, 35-38, 40-41 and 44-50.

The peptides of the invention are active in inhibiting angiogenesis in the in vitro bovine aorta endothelial (BAE) cell proliferation assay with an  $IC_{50}$  of 20  $\mu$ M or less, preferably 5  $\mu$ M or less, more preferably 1  $\mu$ M or less and most preferably below 0.1  $\mu$ M. Much preferred peptides of the  
10 invention are active in the in vitro BAE cell proliferation assay with an  $IC_{50}$  of about 20 pM.

Alternatively, preferred peptides of the invention will exhibit inhibition of angiogenesis in a chick chorioallantoic membrane assay of at least 30% (i.e. inhibition is observed under at least 3 of 10 coverslips  
15 applied) at a dose of 50  $\mu$ g/coverslip. More preferably at least 50% inhibition is observed at a dose of 25  $\mu$ g/coverslip. Most preferably, inhibition in the range of 50-80% is observed for a dose ranging from 10-25  $\mu$ g/coverslip.

Also, the peptides of the invention encompass peptides that have  
20 been derivatized, for example to improve transport across membranes by attachment of hydrophobic groups or that are carboxy-terminal amidated to improve resistance to serum proteases. Methods for accomplishing such derivatizations are known in the art.

Formulation of the peptides for administration is standard in the art.  
25 It is anticipated that optimization of formulation, dosages and schedule of administration is also standard in the art. Reference 16, especially at parts 4, 5 and 7, provides guidance in these matters. Administration of the peptides for anti-tumor treatment can be by any route, preferably oral, intravenous, intradermal, subcutaneous or aerosol. It is expected that  
30 subcutaneous administration is especially effective when anti-metastatic activity is desired (see, reference 6).

The dosage of the peptides will depend upon the therapeutic index of the compound. It is noted that angiostatin has been administered to mice in amounts of 100 mg/kg/day without observable toxicity. Inhibition of metastasis by angiostatin is observed at doses of about 1 mg/kg/day and inhibition of primary tumor growth is observed at about 10 mg/kg/day (6). Thus, it is expected that effective doses of the peptides of the present invention will be about 0.2 µg/kg/day to about 2 mg/kg/day for inhibition of metastasis. Preferred dosages are in the 2-200 µg/kg/day range, most preferred dosages are in the 20-50 µg/kg/day range. It is expected that the dosage ranges will be about 10-fold higher for inhibition of primary tumor growth.

**Example 1:  
Anti-proliferative Activity of Peptides Derived from Angiostatin**

The peptides were tested in an in vitro bovine aorta endothelial cell (BAE) proliferation assay for their ability to inhibit BAE cell proliferation. Cells were plated into 24-well culture plates at  $1.25 \times 10^4$  cells/well in complete SFM-endothelial medium (Gibco, USA). After 24 h, the medium was removed and various amounts of peptides were added into the wells in 0.5 ml of SFM-endothelial medium without bFGF. After 30 minutes incubation, bFGF was added into each well at a final concentration of 1 ng/ml together with another 0.5 ml of SFM-endothelial medium. Seventy-two hours later, cells were trypsinized and counted using a hemocytometer. Commercial E. coli recombinant human angiostatin (1 µM) was used as a control in the experiments.

Peptides Angio-2, Angio-3 and Angio-4, containing sequences from kringles 2, 3 and 4 of human plasminogen (SEQ. ID. Nos. 2, 3 and 11), all inhibited BAE cell proliferation in a dose-dependent manner (Figures 1, 2 and 3). The IC<sub>50</sub> of the three peptides are about 2 mM, 20 nM and 200 µM respectively. It is noted that peptide Angio-3 inhibited BAE cell proliferation with an IC<sub>50</sub> in a range similar to the reported nanomolar range of angiostatin protein (5). In contrast, a randomly scrambled



version of Angio-3 (Angio-3S) in which the same amino acids present in Angio-3 were put in a random order, completely lost angiogenesis inhibition ability (Figure 1). This indicates that the antiangiogenic activity of the peptides are sequence dependent. No obvious cell cytotoxicity was observed by peptide angio-3 at 20 $\mu$ M with BAE cells analyzed by trypan blue staining and microscopic cellular morphology analysis.

The BAE inhibition assay provides a method for determining the anti-angiogenic activity of peptides. For example, peptides Angio-3A, -3B, -3C, -3D, -3E and -3F (SEQ. ID. Nos. 4-9) are peptides in which the six amino acids surrounded by the two prolines are individually mutated into alanine. The contribution of each of the six amino acids between the proline residues to the biological activity of Angio-3 can be determined by testing these peptides.

## **Example 2: Anti-angiogenic Activity of Peptides Derived from Angiostatin**

Since peptides Angio-2, Angio-3 and Angio-4 all inhibited BAE cell proliferation, they were also tested in the chick embryo chorioallantoic membrane (CAM) in vivo angiogenesis assay (7). In this assay, the peptides are coated onto 2-3 mm glass squares cut from microscope cover slips. All three peptides inhibited chick CAM angiogenesis (Figures 4, 5 and 6). Peptide Angio-3 reduced microvessel density at the very low dose of 12 pg/cover slip (the lowest dose tested, comparing to a PBS control). As a positive control, recombinant angiostatin at the dose of 100 ug/cover slip was tested in the same experiment. The amount of inhibition (judging from the extent of reduction in microvessel density) from 100 ug recombinant angiostatin is equivalent to 1.2 ug of Angio-3 peptide. Based on this, we estimated that Angio-3 is at least as potent as angiostatin in inhibiting chick CAM angiogenesis.

At high doses, e.g., 1 mg/cover slip, the blood vessels under the cover slip containing all three peptides died and circulation was stopped.

The color of the blood changed into dark red instead of the bright red of the circulating blood.

The above data demonstrated that Angio-2, Angio-3 and Angio-4, small peptides derived from the sequence of human plasminogen kringle domains 2, 3 and 4 function as potent angiogenesis inhibitors. Importantly, peptide Angio-3 is more potent in inhibiting BAE cell proliferation compared to angiostatin protein and at least as potent as recombinant angiostatin in inhibiting chick CAM angiogenesis.

Cao et al. (12) show that integrity of complete kringle domains is required for anti-angiogenic activity of angiostatin and that a tandem pair of properly folded domains is necessary. Cao et al. (13) also show that, at least with respect to the k5 domain, appropriate disulfide bond formation is necessary for maintaining anti-angiogenic activity. Thus, it is surprising that the exemplified small peptides of the invention, which (1) do not form complete kringle domains and (2) do not contain any cysteine residues and therefore do not form disulfide bonds, have such high activity.

**Example 3:  
Antiangiogenic Activity of Peptides Derived from Endostatin, VEGF and VEGF Receptors**

Peptides were tested in an in vitro bovine aorta endothelial cell (BAE) proliferation assay for their ability to inhibit BAE cell proliferation. Cells were plated into 24-well culture plates at the number of  $1.25 \times 10^4$ /well in complete SFM-endothelial media (Gibco, USA). After 24 hours, the medium was removed and various amounts of peptides were added into the wells in 0.5ml of SFM-endothelial medium without bFGF. After 30 minutes incubation, bFGF was added into each well at a final concentration of 1 ng/ml together with another 0.5 ml of SFM-endothelial medium. Seventy-two hours later, cells were trypsinized and counted using a hemocytometer. Peptides Endo-2, Endo-3 and Endo-4 all inhibited BAE cell proliferation in a dose-dependent manner while peptide Endo-1 has only a slight inhibition effect at high doses (Figure 7). The

IC<sub>50</sub> of the Endo-2, -3 and -4 peptides is estimated to be 0.4  $\mu$ M, 0.3  $\mu$ M and 2  $\mu$ M respectively.

All 4 endostatin-derived peptides were also tested in the chick embryo chorioallantoic membrane (CAM) in vivo angiogenesis assay.

- 5 These peptides reduced microvessel density with various efficacy comparing to a PBS control (Figure 8).

Peptides designed from mammalian VEGF sequences (mVEGF) and Zebrafish VEGF (fVEGF) showed dose-dependent inhibition of CAM angiogenesis (Figures 9,10). Scrambled mutant peptide where the same  
10 amino acids in mVEGF were placed in a random order completely destroyed the angiogenesis inhibition activity of mVEGF peptide.

Two small peptides, hFLT1 and hFLT2, were designed from the second immunoglobulin domain (Ig domain 2) of the extracellular region of FLT-1. The peptide hFLT2 showed very potent angiogenesis inhibition  
15 activity on CAM (Figures 10 and 11). Surprisingly, a truncated version of hFLT2 peptide, hFLT2-11, in which the N-terminal two amino acids have been deleted, showed a even more potent antiangiogenic activity comparing to hFLT2 (Figure 11). However, a single amino acid mutated  
20 version of the same peptide, hFLT2-T, in which the second amino acid proline was replaced with threonine, has a much weaker antiangiogenic activity (Figure 11). Peptide hFLT2-9 in which four amino acids were removed from the N-terminal of hFLT2, showed similar level of antiangiogenic activity as the original peptide (Figure 11). Further  
25 truncation at the N-terminal of the peptide resulted in non-functional peptide (hFLT2-7 and hFLT2-5).

The above data demonstrated that small peptides derived from the sequence of human endostatin, VEGF and FLT-1 can function as potent angiogenesis inhibitors.

**Example 4:****Inhibition of endothelial cell proliferation and retardation of tumor growth in mice****Mouse tumor assays**

Human hepatoma cell line HepG2 was cultured and 1 million cells were injected subcutaneously into the right back of each 6 weeks old BALB/c nude mouse. Visible tumors appear from about 2 weeks after tumor cell injection. Mice with tumors were then randomly grouped and injected with the peptide at 300 µg/mouse every 12 h (16-19 g average weight). No obvious toxicity was observed at this dose. Tumor sizes were measured with a caliper every three days and volumes calculated using the formula (width)<sup>2</sup> x length x 0.52. Statistical analyses were done using student t-test using the SigmaStat software (SPSS, USA).

**Peptide angio-3 inhibits tumor growth in nude mouse**

Tumors are angiogenesis dependent. To test if peptide angio-3 can inhibit tumor growth in vivo, human hepatoma HepG2 cells were injected subcutaneously into nude mice to induce tumor formation. Tumor nodules appear in about 15-18 days after tumor cell injection. Peptide was then injected at a distant site from the tumor into tumor bearing mice at 15 mg/kg every 12 h for up to a month. As shown in Fig. 12, injection of peptide Angio-3 inhibited HepG2 tumor growth by about 40% at the time of experimental termination. Experiments were terminated when the control group tumors started to show necrosis. Statistical analysis indicates that the tumor size difference between the treatment and control groups is significant at  $p < 0.1$ .

In summary, this Example demonstrates that angio-3 can inhibit endothelial cell proliferation and retard tumor growth in mice.

## REFERENCES:

The following articles of the periodical and patent literature are cited above. Each text is hereby incorporated in its entirety by reference by such citation.

1. Folkman, J. et al., Cell 87, 1153-1155, 1996 and U.S. Patent 5,639,735.
2. Folkman, J. et al., J. Nature Medicine 1, 27-31, 1995.
3. Kim, K. J. et al., Nature 362, 841-844, 1993.
4. Auerbach, W. et al., Pharmac. Ther. 63, 265-311, 1994.
5. O'Reilly, M. S. et al., Cell 79, 315-328, 1994.
6. O'Reilly, M. S. et al., Nature Medicine 2, 689-692, 1996.
7. O'Reilly, M. S. et al., Cell 88, 277-285, 1997.
8. Boehm, T. et al., Nature 390, 404-407, 1997.
9. Kerbel, R. S., Nature 390, 335-336, 1997.
10. Handin, R. I. et al., Chapter 58 of "Heart Diseases: A Textbook Of Cardiovascular Medicine," 4<sup>th</sup> ed. c. 1992 by W. B. Saunders Company, pp. 1767-1789.
11. O'Reilly, M. S. et al., U.S. Patent 5,639,725, 1997.
12. Cao, Y. et al., J. Biol. Chem. 271, 29461-29467, 1996.
13. Cao, Y. et al., J. Biol. Chem. 272, 22924-22928, 1997.
14. Kini, R. M. et al., Curr. Topic Peptides & Prot. Res. 1, 297-311, 1994.
15. Kini, R. M. et al., Biochem. Biophys. Res. Comm. 212, 1115-1124, 1995.
16. "Remington: The Science and Practice of Pharmacy", 19<sup>th</sup> ed., c. 1995 by the Philadelphia College of Pharmacy and Science.
17. Oh, S. P. et al., PNAS USA 91, 4229-4233, 1994.
18. Rehn, M. et al., PNAS USA 91, 4234-4238, 1994.
19. Muragaki, Y. et al., PNAS USA 92, 8763-8767, 1995.
20. Ding, Y-H et al., PNAS USA 95, 10443-10448, 1998.
21. Boehm, T. et al., Biochem. Biophys. Res. Comm. 252, 190-194, 1998.

22. Ferrara, N. et al., *Endo. Rev.* 18, 4-25, 1997.
23. Keyt, B. A. et al., *J. Biol. Chem.* 271, 5638-5646, 1996.
24. Muller, Y. A. et al., *PNAS USA* 94, 7194-7197, 1997.
25. Muller, Y. A. et al., *Structure* 5, 1325-1338, 1997.
26. Weismann, C. et al., *Cell* 91, 695-704, 1997.
27. Barleon, B. et al., *J. Biol. Chem.* 272, 10382-10388, 1997.
28. Cunningham, S. A. et al., *Biochem. Biophys. Res. Comm.* 231, 569-599, 1997.
29. Millauer, B. et al., *Nature* 367, 576-579, 1994.
30. Kong, H. et al., *Human Gene Therapy* 9, 823-833, 1998.
31. Risau, W. Mechanisms of angiogenesis. *Nature* 1997; 386: 671-674.
32. Ji, W-R., Barrientos, L. G., Llinas, M., Gray, H., Villarreal, X., Deford, M. E., Castellino, F. J., Kramer, R. A and Trail, P. A. Selective inhibition by kringle 5 of human plasminogen on endothelial cell migration, an important process in angiogenesis. *Biochem. Biophys. Res. Commun.* 1998; 247: 414-419.
33. Lu, H., Dhanabal, M., Volk, R., Waterman, M. J., Ramchandran, R., Knebelmann, B., Segal, M and Sukhatme, V. P. *Biochem. Biophys. Res. Commun.* 1999; 258: 668-673.